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VISIONS AND PERSPECTVES

The importance of studying invertebrate immune-neuroendocrine functions

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Abstract

The present paper illustrates various advantages of the use of invertebrates as an experimental model as these models provide important evidence in the field of evolutionary research and at the same time offer practical applications in the bio-medical and agronomy fields of research.

Key Words: invertebrate; evolution; translational biology

Introduction

Invertebrates approximately represent 96 % of all animal species, and thanks to their excellent capacity to adapt they can be found in almost every habitat of the Earth, from the coldest areas, such as the poles to the warmest, such as the deserts. This unique combination of diversity and simplicity represent a great advantage for the study of defense mechanisms. However, caution must be taken when citing the "simplicity of the model", because nowadays it is well known that invertebrate systems, though simpler than those of vertebrates, are far from being simple. As far as the immune-neuroendocrine system is concerned, my group as well as others have revealed the involvement of numerous mediators in what that include mammalian-like molecules such as the neurohormone corticotrophin releasing hormone (CRH); proopiomelanocortin products such as corticotropin (ACTH) and β-endorphin; small bioactive molecules such as norepinephrine, epinephrine and dopamine, steroid hormones such as glucocorticoids, cytokines such as IL-1, IL-6 and TNF-α and gaseous mediators such as nitric oxide (Ottaviani *et al*., 1997).

Why study invertebrate immune-neuroendocrine functions?

By analyzing the changes that different species have undergone in time, evolutionary studies highlight changes at the molecular level, as well as the mechanisms that allowed the accumulation of molecular variants and selection. But while changes have been found to be important we can now assert on the basis of our previous data (Ottaviani and Franceschi, 1996; Ottaviani *et al*., 1997) as well as the molecular data now available in the literature on all the major families of proteins (antibodies, adhesion molecules, heat shock molecules, calcium-binding molecules, etc.) that conserved traits are highly significant and, in some cases, more relevant than changes. Our research on the evolution of signaling molecules in immune-neuroendocrine functions (*e.g.,* neuropeptides, hormones and cytokines) have revealed that these primary signaling molecules are conserved very well across species. Despite the progressive modification of organs and systems, the mechanisms which govern the exchange of information between cells have been maintained.

In this context, the molecular diversity of complex biological systems, such as the immune system seems to be derived from a single ancestral protein domain which by duplication, gene conversion, DNA recombination, rearrangement and other molecular mechanisms have generated all the molecules of the immune system (Marchalonis and Schluter, 1994).

Immunocytochemistry, flow cytometry, RIA tests and functional studies of some molecules in various invertebrate species show the presence of ACTH and β-endorphin-like molecules in cells with macrophage characteristics (Ottaviani *et al*., 1997). Such molecules have a physiological role in chemotaxis and phagocytosis, and the role of ACTH appears to be broader than that of β-endorphin.

With respect to the neuroendocrine response, the results obtained in molluscs demonstrate that stress activates an axis that leads to the release of biogenic amines from immunocytes upon activation of both the CRH and ACTH-dependent cascade (Ottaviani and Franceschi, 1996). Overall, we are witnessing the presence of an axis CRH-ACTH-

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biogenic amines as seen in vertebrates, which is even more notable, considering the observation that in invertebrates, in contrast to vertebrates, organs such as the hypothalamus, the pituitary and the adrenal gland are not present.

Moreover, recent data indicate that the transcriptional response of neurons to immunological stress in a mollusc, involves epigenetic modifications just like in vertebrates. Here epigenetic changes occurred in the freshwater snail *Pomacea canaliculata* after injection with LPS in form of immunopositivity towards phospho (Ser10)-acetyl (Lys14)-histone H3 and enhanced c-Fos in the nuclei of small gangliar neurons. Western blot analysis revealed a significant increase of phospho (Ser10)-acetyl (Lys14)-histone H3 in nuclear extracts 2 h after receiving an injection with LPS. c-Fos protein levels were significantly enhanced 6 h after LPS injection (Ottaviani *et al*., 2013).

Another example of conserved immune-neuroendocrine response is provided by cytotoxicity, present throughout metazoans. There is a remarkable conservation of a cytotoxicity type NK-like cell from invertebrates to man. For instance, cells of mollusc were able to lyse target cells K562 pre-labeled with ^{51}Cr (Franceschi *et al*., 1991).

The components of signal transduction pathways in the modulation of the innate immune response have been highly conserved during evolution. Kim and Ausubel (2005) report that the TIR-1 (Toll/IL-1receptor)/SARM (sterile alpha and HEAT/armadillo motif)-PMR (p38 mitogen-activated protein kinase family)-1/p38 MAPK (MAP kinase) pathway is the ancestral immune signaling pathway of the common ancestor of nematodes, arthropods and vertebrates.

On the whole, the findings reported above display a close parallelism between invertebrates and vertebrates in terms of molecules and of signaling pathways and this provides a valuable tool for studying the evolutionary mechanisms underpinning diversification of multicellular organisms.

Beside the interest for basic research this conservation presents potential application for translational research. For instance, in the bio-medical research field, the isolation and purification of invertebrate immune-related molecules may provide new mediators in models for the development of new chemotherapeutic agents. Furthermore, in agronomy, biological control praxes may find in invertebrate molecules potential factors for the prevention of the disasters caused by pests in agriculture.

Studies performed in marine invertebrates, *i.e.*, sponges and ascidians, have demonstrated their capacity to produce secondary metabolites with unique structures and potent bioactivities providing an important source of new drugs for human and animal diseases and in particular against cancer (Sandler, 2005; Imperatore *et al*., 2012). Peptides purified from sponges, ascidians, tunicates and molluscs have an antioxidant activity and cytotoxic effect on several human cancer cell lines such as HeLa, AGS and DLD-1 (Suarez-Jimenez *et al*., 2012). The biological activities of the secondary metabolites with anticancer and cytotoxic properties are exerted on the cytoskeleton, which plays a central role in cellular proliferation, motility and is deeply involved in the metastatic process associated with tumors (Mollica *et al.,* 2012). Many anticancer substances isolated from sponges are already in clinical use or undergoing the final stages of clinical trials (Laport *et al*., 2009).

Antimicrobial peptides such as cecropin A and B from the silk moth *Cecropia* are able to inhibit the growth of and to kill yeast-phase *Candida albicans* (Andrä *et al.*, 2001). Moreover, a cecropin isolated from *Musca domestica* is a bactericidal agent against clinical isolated multidrug-resistant *Escherichia coli* (Lu *et al*., 2012).

In agriculture, several strategies have been developed for insect control, includes mating disruption (MD), pheromone antagonists, pheromones and plant-based volatiles, attractant-and-kill, and push-pull strategies (Reddy and Guerrero, 2010).

Many insect species communicate with each other by a variety of chemical signals called pheromones. In particular, sex pheromones attract one sex to the other so that mating can take place, a phenomenon common in the insect order of Lepidoptera. Sex pheromones are a complex mixture of chemicals and each species has it own specific blend. MD technology uses synthetic products closely related to the pheromones produced by the female (known as a “calling” female) that are able to confuse males and limit their ability to locate calling females. For instance, the SPLAT-OrB, a pheromone formulation, provokes MD in the oriental beetle (Rodriguez-Saona *et al*., 2010). Another interesting study on MD was conducted in Guatemalan potato moth *Tecia solanivora* using the following sex pheromone components: (E)-3-dodecenyl acetate, (Z)-3-dodecenyl acetate, and dodecyl acetate (McCormick *et al.,* 2012).

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